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## Effect of postpartum suppression of ovulation on uterine involution in dairy cows

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**Effect of postpartum suppression of ovulation on uterine involution in dairy cows**

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**Abstract**

The objective of this study was to investigate the effect of time of first postpartum ovulation after calving on uterine involution in dairy cows with and without uterine puerperal disease. Transvaginal follicular puncture (FP) of follicles  $> 6$  mm suppressed ovulation and development of a CL until Day 42 after calving. Fifty-three lactating Holstein Friesian cows ( $3.4 \pm 1.2$  yr old, parity  $2.5 \pm 1.0$  [median  $\pm$  mean absolute deviation]) were divided into groups based on the presence (UD+) or absence (UD-) of uterine disease and whether follicular puncture was carried out (FP+) or not (FP-). Uterine disease was defined as the occurrence of retained fetal membranes and/or metritis. This resulted in the following groups: UD-FP- ( $n = 15$ ), UD-FP+ ( $n = 13$ ), UD+FP- ( $n = 13$ ), and UD+FP+ ( $n = 12$ ). A general examination, vaginoscopy, transrectal palpation, and transrectal B-Mode sonography of the reproductive organs were conducted on Days 8, 11, 18, 25 and then every ten days until Day 65 after calving. After hormonal synchronization of ovulation (cloprostenol between Days 55 and 60 postpartum and GnRH 2 d later), cows were inseminated in the next spontaneous estrus. On average, the cows ovulated on Day  $21.0 \pm 6.0$  (UD-FP-),  $50.0 \pm 4.0$  (UD-FP+),  $16.0 \pm 3.0$  (UD+FP-), and  $48.0 \pm 2.0$  (UD+FP+) postpartum. Calving-to-conception interval and first-service conception rates were not affected by FP ( $P > 0.05$ ). Healthy cows with FP had smaller ( $P < 0.05$ ) uterine horn and cervical diameters assessed sonographically than cows without FP. Follicular puncture reduced the prevalence of purulent vaginal discharge and uterine size assessed transrectally in UD+ cows ( $P < 0.05$ ). The results showed that suppression of an early ovulation by transvaginal follicular puncture improved uterine involution in cows with and without uterine disease.

Keywords: Puerperium, uterine disease, progesterone, ultrasonography, transvaginal follicular puncture, cattle

## 1. Introduction

The puerperal period is a critical phase in the reproductive cycle of dairy cows (5). Uterine involution, which includes reduction of uterine size, regeneration of the endometrium, elimination of bacterial contamination and resumption of ovarian cyclicity occurs during this period and is a prerequisite for subsequent gestation (29). The calving-to-first ovulation interval is affected by nutrition, body condition (24), suckling (30), parity (36), and uterine disease (27,38). In healthy cows the interval to first ovulation lasts from 21 to 30 days (12,23,25). Puerperal uterine diseases may prolong the anovulatory interval by up to ten days (39).

Published information on the effect of length of the postpartum anovulatory period on reproductive performance in cows is equivocal. Some studies have shown that shortening of the postpartum anovulatory period is associated with improved fertility (3,6,13,37), whereas others reported reduced fertility (34) or did not find a positive effect (23). The latter phenomenon was explained by immunosuppression and muscle relaxation attributable to progesterone ( $P_4$ ) from the corpus luteum during the early postpartum period (1,2). Furthermore, early ovulation may be associated with luteal persistence if accompanied by bacterial uterine contamination (4,16), possibly related to preferential accumulation of luteotropic PGE, rather than luteolytic PGF, in cows with an inflamed endometrium (28).

Previous experimental studies on the effect of the postpartum anovulatory interval on fertility of dairy cows have used estrogens or GnRH analogues (deslorelin) for postpartum suppression of ovulation (11,18,31-33). Whether and to what degree uterine effects were caused indirectly by the suppression of ovulation or directly by the experimentally used hormones is not known. Estrogens have a positive effect on myometrial contractility and enhance uterine immune response (14,22,31). GnRH analogues also enhance uterine motility (7). One study on the effect of the timing of the first postpartum ovulation on uterine involution included cows with and without puerperal uterine diseases but results were not analyzed by the health status of the cows (33).

Transvaginal follicular puncture provides an experimental method of postpartum suppression of ovarian activity without the use of hormones. This technique is well tolerated by cows; new cyclic ovarian activity starts as early as 4 to 7 d after follicle aspiration, and negative effects on ovarian structure and function are minimal (8,19,35).

The aim of this study was to investigate the effect of postpartum suppression of ovulation achieved by repeated transvaginal follicular puncture on uterine involution in healthy dairy cows and in cows with postpartum uterine disease.

## **2. Material and Methods**

### *2.1. Animals*

This study was carried out at the Research Farm of the University of Veterinary Medicine Hannover, Germany, between February 2009 and July 2010 and was

approved by, and in accordance with, German legislation on animal rights and welfare (33.9-42502-04-08/1592). The herd consisted of 90 milking Holstein Friesian cows and the rolling herd average was 10 100 kg of milk per lactation. The cows were housed in a freestall barn and fed a total mixed ration supplemented with concentrate based on milk yield. Fresh water was available *ad libitum*.

## 2.2. Study design

Between Days 4 and 21 after calving (Day 0 = day of calving) the animals were divided into the following two groups: cows with no uterine disease (UD–) and cows with uterine disease (UD+). Uterine disease was defined as the occurrence of retained fetal membranes (membranes not shed within 24 h after calving) and/or metritis. Metritis was diagnosed by transrectal palpation and vaginal examination according to Sheldon et al. (26). Cows of both groups were randomly subdivided into two subgroups: in the first, ovulation was suppressed by repeated transvaginal follicular puncture (FP) until Day 42 (group FP+) and in the second, FP was not carried out (group FP–). This resulted in the four groups designated UD–FP–, UD–FP+, UD+FP–, and UD+FP+. Cows from the FP– groups that did not ovulate by Day 42 and cows from the FP+ groups that ovulated or had a progesterone (P<sub>4</sub>) concentration > 1.0 ng/ml before Day 42 were excluded from the study. Ovulation was diagnosed retrospectively when a CL (> 20 mm) was detected ultrasonographically. Cows with other diseases (lameness, digestive disorders, mastitis) during the study period were also excluded.

Cows underwent a general examination, vaginoscopy and transrectal palpation, and B-Mode sonography of the reproductive tract on Days 8, 11, 18, 25 and then every

ten days until Day 65. For synchronization of ovulation all cows received intramuscular injections of 0.5 mg cloprostenol (Estrumate<sup>®</sup>, Intervet, Unterschleißheim, Germany), between Days 55 and 60, and 0.01 mg GnRH (buserelin; Receptal<sup>®</sup>, Intervet, Unterschleißheim, Germany) 2 d later. AI was performed in the following spontaneously occurring estrus. Pregnancy diagnosis was conducted sonographically 35 to 50 d after AI. Detection of an embryo with a heartbeat was taken as evidence for pregnancy. Calving-to-first ovulation interval, calving-to-conception interval, and first-service conception rate were calculated for evaluation of reproductive performance.

### *2.3. Transrectal palpation and vaginoscopy*

Transrectal palpation served to assess uterine size and contractility. Uterine size was classified according to Grunert (10) and each cow was allocated to one of six categories: uterus retractable and horn diameter < 2 cm (1), 2 to 5 cm (2) or > 5 cm (3), uterus not retractable but greater curvature palpable (4), uterus not retractable and greater curvature incompletely palpable (5), and uterus not retractable and greater curvature poorly outlined (6). Uterine contractility was classified as absent (0), moderate (1), or strong (2).

A tube speculum and flashlight were used for vaginoscopy. During the early postpartum period ( $\leq 21$  d postpartum), vaginoscopy was used to classify cows as UD+ and UD- according to Sheldon et al. (26). After Day 21 postpartum, the vaginal discharge was categorized as absent (0), clear mucus (1), mucus containing flecks of pus (2), up to 50% pus (3), or more than 50% pus (4; from Sheldon et al (26), modified).

## 2.4. Ultrasonography

Transrectal B-Mode sonography was carried out with a Powervision SSA-370 ultrasound machine (Toshiba Co., Tokyo, Japan) equipped with a 7.5-MHz microconvex transducer. Cross-sectional images of the cervix and uterine horns were obtained by placing the transducer accordingly. The middle part of the cervix was imaged and the uterine horns were evaluated approximately 2 cm cranial the bifurcation. Three images were taken at each position, frozen, and stored on a Magneto Optical Disc (Sony, Tokyo, Japan). Analysis was done off-line on a personal computer using FixFoto<sup>®</sup> software (Version 2.74, Joachim Koopmann Software, Wrestedt-Stederdorf, Germany). The best images were selected for measurement of the cervical and uterine horn diameters. The former was defined as vertical dimension from serosa to serosa through the center of the organ. Because the cross-sectional image of the uterine horns was often oval, diameter was calculated as the mean of the maximum height and width of the endometrium. The ovaries were examined for follicles and CLs.

## 2.5. Transvaginal follicular aspiration

At the start of the study all follicles  $\geq 10$  mm were punctured twice a week. However, because several cows ovulated and had to be excluded from the study (see 3.), the FP interval was reduced to two or three days and all follicles  $\geq 6$  mm were punctured. Epidural anesthesia using 3 mL procaine hydrochloride (Procasel<sup>®</sup> 2%, Selectavet, Weyarn-Holzolling, Germany) was administered and the ovaries identified using a



Logiq Book XP ultrasound machine (General Electrics Medical Systems, Jiangsu, P. R. China) equipped with a 7.5-MHz microconvex transducer, which was inserted vaginally. The transducer and the aspiration needle (Sterican<sup>®</sup> 0.90 x 50 mm, B. Braun Melsungen AG, Melsungen, Germany) were encased in a PVC carrier, which was protected by a latex cover (MAPA<sup>®</sup>, Zeven, Germany) filled with ultrasound gel (Waldeck GmbH & Co KG, Münster, Germany). The ovaries were fixed transrectally by hand, the needle inserted into the follicles to be aspirated, and the fluid aspirated using a syringe and plastic tubing (17).

## *2.6. Steroid hormones*

Blood samples were collected weekly from a jugular vein into EDTA tubes and placed on ice after collection. After centrifugation (2000 x g, 20 min at 4°C), plasma was separated and stored at -20°C until analysis. The plasma P<sub>4</sub> concentration was measured using a commercial coat-a-count radioimmunoassay according to the manufacturer's instructions (Progesterone Coat-a-Count, TKPG1, Siemens Medical Diagnostics, CA, USA). The analytical sensitivity was 0.02 ng/ml, the intra-assay coefficient of variation (CV) was 4.0%, and the inter-assay CV was 7.6%. Total estrogen (E) concentration was determined after ether extraction with a direct enzyme-immunoassay on microtiter plates using a secondary-antibody coating technique and horseradish peroxidase as the enzyme label (15,21). The antiserum used was raised against 17 $\beta$ -estradiol hemisuccinate (cross reactivity 17 $\beta$ -estradiol 100%, estrone 100%, 17 $\alpha$ -estradiol 70%). The 17 $\beta$ -estradiol hemisuccinate horseradish peroxidase was used as steroid-enzyme conjugate. The minimal

detectable concentration was 8 pg/ml. Recovery ranged from 84.6 to 96.2%, the intra-assay CV was 13.2%, and the inter-assay CV was 19.7%.

### *2.7. Statistical analysis*

Statistical analyses were carried out using the Statistical Analysis System (SAS Institute, Cary, North Carolina). The Shapiro-Wilk test was used to test for normality of the distribution of all variables. Because not all variables were normally distributed, median and median absolute deviation (MAD) values were given. Differences between groups were analyzed using the non-parametric Wilcoxon's rank sum test (PROC NPAR1WAY). The two variables time of ovulation and length of first cycle were compared between the UD– and UD+ groups, and all other variables were only compared between the FP– and FP+ groups. The effect of day postpartum on variables was determined using the Friedman two-way ANOVA (PROC FREQ). Differences in values between Days 4 and 25 and between Days 25 and 65 within groups were analyzed using the Wilcoxon's signed rank test (PROC UNIVARIATE) and differences between groups were analyzed using a combination of Wilcoxon's signed rank test (PROC UNIVARIATE) and Wilcoxon's rank sum test (PROC NPAR1WAY). The  $\chi^2$ -test of homogeneity was used to compare categorical data between groups (PROC FREQ). Differences were considered significant at  $P < 0.05$ .

## **3. Results**

A total of 83 cows were included in this study; six were excluded because of displaced abomasum ( $n = 2$ ), mastitis ( $n = 2$ ), severe lameness ( $n = 1$ ), and

reticuloperitonitis ( $n = 1$ ). Fifteen cows of the FP+ group ovulated before Day 42, and three cows without sonographic evidence of ovulation had  $P_4$  concentrations greater than 1.0 ng/ml in the first 42 d. Six cows of the FP– group did not ovulate in the first 42 d (UD–,  $n = 2$ ; UD+,  $n = 4$ ). This resulted in the following group sizes: UD–FP–,  $n = 15$ ; UD–FP+,  $n = 13$ ; UD+FP–,  $n = 13$ ; UD+FP+,  $n = 12$ . The cows were  $3.4 \pm 1.2$  yr old, parity was  $2.5 \pm 1.0$ , and BCS was  $2.75 \pm 0.25$ . The groups did not differ ( $P > 0.05$ ) with respect to these variables.

Table 1 shows time of first ovulation, first-cycle length, calving-to-conception interval, and first-service conception rate for each group. The UD+FP– and UD–FP– groups differed with respect to first-cycle length ( $P < 0.05$ ).

Table 1 here

### *3.1. Transrectal palpation and vaginoscopy*

Uterine size was affected by time ( $P < 0.0001$ , Fig. 1); the score decreased ( $P < 0.05$ ) by 67% (UD–FP–), 60% (UD–FP+ and UD+FP+), and 40% (UD+FP–) in the first 25 d. Thereafter until Day 65 it declined further ( $P < 0.05$ ) by 17% (UD–FP–), 20% (UD–FP+ and UD+FP+) and 40% (UD+FP–). There was a trend ( $P = 0.07$ ) in the UD+ group for a smaller decrease in uterine size between Days 25 and 65 in FP+ cows compared with FP– cows. In the UD+ group there was an effect of FP on uterine size ( $P < 0.05$ ). On Day 4, UD–FP– cows had greater ( $P < 0.05$ ) uterine size than UD–FP+ cows. On Days 11, 18, 25 and 35, UD+FP– cows had larger ( $P < 0.05$ ) uterine size than UD+FP+ cows. Uterine contractility was affected by time in the UD–FP+ and UD+FP– cows ( $P < 0.05$ ), but not by FP within groups ( $P > 0.05$ ).

Figure 1 here

The incidence of vaginal discharge was affected by time in UD+ cows ( $P < 0.05$ ; Table 2) but not in UD– cows ( $P > 0.05$ ). There was an effect of FP in UD+ cows ( $P < 0.05$ ); on Day 35, more UD+FP– cows ( $P < 0.05$ ) had vaginal discharge compared with UD+FP+ cows.

Table 2 here

### *3.2. Ultrasonographic findings*

Cervical diameter was affected by time in all groups ( $P < 0.0001$ ; Fig. 2). Between Days 8 and 25, the diameter decreased ( $P < 0.05$ ) by 33% (UD–FP– and UD+FP+), 38% (UD–FP+), and 31% (UD+FP–). After Day 25, it decreased ( $P < 0.05$ ) by another 24% (UD–FP–), 10% (UD–FP+), 12% (UD+FP–), and 16% (UD+FP+). In group UD– there was a trend for a smaller decrease ( $P = 0.07$ ) in cervical diameter between Days 25 and 65 in FP+ cows compared with FP– cows. For the UD– animals an effect of FP could be noticed ( $P < 0.05$ ). On Days 25, 35, and 65, UD– cows with FP had a smaller ( $P < 0.05$ ) cervical diameter than cows without FP. In UD+ cows, the same effect occurred on Day 65 ( $P < 0.05$ ).

Figure 2 here

Diameter of both horns was affected by time in all groups ( $P < 0.0001$ ; Fig. 3). By Day 25, the diameter of the previously pregnant/non-pregnant horns decreased ( $P < 0.05$ ) by 55%/35% (UD–FP–), 55%/45% (UD–FP+), 57%/43% (UD+FP–), and 52%/42% (UD+FP+). After Day 25, diameters decreased ( $P < 0.05$ ) by 8%/8% (UD–FP–) and 7%/10% (UD+FP–) or the decrease was merely numerical (3%/0%, UD–FP+; 7%/8%, UD+FP+;  $P > 0.05$ ). In the UD– group, FP+ cows had a smaller decrease in horn diameter than FP– cows between Days 25 and 65 ( $P < 0.05$ ). Over the whole examination period UTD-values were not affected by FP within UD groups ( $P > 0.05$ ). Only on Day 18 the diameter of both uterine horns were larger ( $P < 0.05$ ) in the UD–FP— cows compared with UD–FP+ cows (Fig. 3).

Figure 3 here

Five UD+FP– cows, but no cows of other groups, developed a persistent CL (38%;  $P < 0.05$ ).

### 3.3. Steroid hormone concentrations

Plasma  $P_4$  concentrations were affected by time in all groups ( $P < 0.0001$ ; Fig. 4). In UD–FP– cows they exceeded 1.0 ng/ml between Days 21 and 28 and in the UD+FP– cows between Days 14 and 21. In FP+ cows a  $P_4$ -increase above 1.0 ng/ml occurred between Days 49 and 56. In FP– cows the  $P_4$  concentrations decreased below 1.0 ng/ml between Days 49 and 56. FP group affected  $P_4$  concentration in UD– and UD+ cows ( $P < 0.05$  and  $P < 0.001$ ). In the UD– group, FP– cows had higher ( $P < 0.05$ )  $P_4$  concentration than FP+ cows on Days 35 and 42. The UD+FP– cows

had higher ( $P < 0.05$ )  $P_4$  concentrations on Days 21, 28, 35, 42, and 49 than UD+FP+ cows.

Figure 4 here

There were no effects of time and FP on total E concentration ( $P > 0.05$ ). On Day 21, UD–FP– cows had higher and on Day 49 had lower ( $P < 0.05$ ) E concentrations than UD–FP+ cows (Fig. 5).

Figure 5 here

#### **4. Discussion**

Suppression of ovulation by transvaginal FP had a positive effect on uterine involution in healthy cows as well as in cows with uterine disease. Healthy cows with FP had smaller cervical diameter and a faster reduction of uterine horn diameters than cows without FP, and in cows with postpartum disease, FP was associated with smaller uterine size and lower incidence of vaginal discharge compared with cows without FP. This supports the idea that postpartum suppression of ovulation, and thus secretion of progesterone, enhances reduction in uterine size and the elimination of inflammation and bacterial contamination, especially in cows with uterine disease. Suppression of early postpartum ovulation in cows using slow-release deslorelin implants also resulted in smaller cervical and uterine diameters compared with untreated controls (31,33). In contrast to those studies, uterine contractility was not affected by the time of the first ovulation in our experiment.

Progesterone has a relaxing effect on the uterus (1,22) and therefore we expected FP to be associated with increased uterine contractility. In cows treated with deslorelin, the effect on uterine and cervical size, seems to be more pronounced than in the present study (31,33). A possible reason for this can be the intrinsic positive inotropic effect of deslorelin on the uterus (7); cows treated with this drug had increased uterine tone compared with untreated cows (31,33). Another possible explanation for the discrepancy between studies can be that intravaginal manipulations and epidural anesthesia during FP had an adverse effect on uterine involution.

Purulent vaginal discharge was more common in UD+FP– cows than in UD+FP+ cows. This could be associated with the immunosuppressive effect of progesterone (2,14) resulting in impaired elimination of bacterial contamination and inflammation in the postpartum period. Purulent vaginal discharge was uncommon in healthy cows as expected, and unlike cows treated with deslorelin (31), we did not observe an increase in vaginal discharge in FP+ cows compared with FP– cows. However, the definition of healthy cows may have differed between studies because previously (31), only cows with retained placenta, dystocia, and stillbirth were excluded.

Interestingly, in healthy cows, FP was associated with a significant reduction in uterine horn diameter only on Day 18. In addition to the positive influence of FP, elevated estrogen concentrations in the FP– cows could have contributed to the difference. High levels of estrogens during estrus lead to uterine edema and thus to an increase in uterine size (12,20). Possible differences in concentrations of total estrogens between FP+ and FP– animals, might have been missed in the present study, because of the low frequency of blood sampling.

After Day 25, the uterine horn diameter measured sonographically decreased only minimally (Fig. 3), whereas the score for uterine size assessed transrectally continued to decrease (Fig. 1). During transrectal palpation, uterine length was estimated in addition to uterine diameter, and uterine diameter decreases more quickly than uterine length (9). Because of the length of the uterus, overall uterine size may be estimated to be larger via transrectal palpation than by merely measuring the cornual cross-sectional diameter near the bifurcation. When the uterus is enlarged by fluid accumulation, which tends to collect toward the tips of the horns, measurement of the cornual diameter is likely to underestimate the overall uterine size.

All analyzed FP+ cows had progesterone concentrations below 1.0 ng/ml until Day 49. Three FP+ cows were excluded from the study because of progesterone concentrations exceeding 1 ng/ml within the first 42 d after calving even though ovulation was not detected sonographically. Based on the results of other studies (19,35), we assumed that these cows generated luteal tissue from residual tissue of punctured follicles. This luteal tissue does not reach the size of a cyclic CL but is active endocrinologically and produces near-physiologic mid-cycle progesterone concentrations (19). In agreement with earlier studies (19,35), FP+ cows ovulated 6 to 8 d after the last follicle aspiration. This procedure interferes with the ovarian follicular dynamics and follicular growth and is followed by rapid resumption of cyclic activity. FP removes the inhibitory effects of estradiol and inhibin on FSH, and the initiation of a new cohort of follicles and the growth of a dominant follicle are enhanced (19). In contrast, the first ovulation after suppression of ovarian activity with deslorelin from Days 7 to 35 occurred 12 d after removal of the GnRH implant (18).



Cows not undergoing FP ovulated and had progesterone concentrations greater than 1.0 ng/ml in the first three weeks. Persistent CLs only occurred in UD+FP– cows, which was in agreement with previous reports that early postpartum ovulations accompanied by uterine infection are prone to lead to luteal persistence (16,28). Inducing cyclic activity in the early postpartum period with GnRH has been proposed for improvement of fertility in dairy herds (2,16), although this practice was associated with an increased incidence of pyometra (4). Taken together, these findings advocate against early induction of ovulation in cows with postpartum uterine disease.

There was no significant effect of time of first postpartum ovulation on reproductive performance. This was in agreement with an earlier report (23) but in contrast to another study, in which early ovulation was associated with a reduced fertility in multiparous cows (34). However, FP+ cows had numerically longer calving-to-conception intervals, similar to cows that received continuous deslorelin treatment for 28 d to delay ovulation. In the latter study, the effect could have been due to delayed onset of cyclic activity because the time from first ovulation to conception did not differ between the treated and untreated cows (18). Furthermore, pregnancy rates did not differ either, which does not support an adverse effect of an extended postpartum anovulatory period on reproductive performance (18), and in the present study, conception rate to first service was slightly increased numerically in UD–FP+ cows compared with UD–FP– cows. However numbers of cows in this study were not sufficient to draw meaningful conclusions with respect to reproductive performance.

In conclusion, suppression of early ovulation and luteal activity by transvaginal FP improves uterine involution in healthy cows as well as in cows with uterine disease. Because of a high incidence of luteal persistence after early ovulation in cows with

397 uterine disease, early hormonal induction of cyclic activity is not recommended in  
398 these cows.

399

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**Tables**

Table 1: Time of first ovulation, first-cycle length, calving-to-conception interval, and first-service conception rate in cows with and without uterine disease (UD+/UD–) and with and without follicle aspiration (FP+/FP–) (medians  $\pm$  MAD).

	<b>UD–FP– (n=15)</b>	<b>UD–FP+ (n=13)</b>	<b>UD+FP– (n=13)</b>	<b>UD+FP+ (n=12)</b>
<b>Time of first ovulation (days postpartum)</b>	21.0 $\pm$ 6.0	50.0 $\pm$ 4.0	16.0 $\pm$ 3.0	48.0 $\pm$ 2.0
<b>First-cycle length (days)</b>	19.5 $\pm$ 2.0 <sup>a</sup>		25.0 $\pm$ 5.0 <sup>b</sup>	
<b>Calving-to-conception interval (days)</b>	107.0 $\pm$ 26.0	120.0 $\pm$ 27.0	119.0 $\pm$ 22.0	135.5 $\pm$ 23.0
<b>First-service conception rate (%)</b>	40	46	39	25

Values with letters (a, b) indicate differences between groups UD–FP– and UD+FP– (P<0.05).

541 Table 2: Relative and absolute incidence of purulent vaginal discharge in cows with  
 542 and without uterine disease (UD+/UD–) and with and without follicle aspiration  
 543 (FP+/FP–) at different examination times postpartum

Group	Days postpartum				
	25	35	45	55	65
<b>UD–FP–</b>	7.1% (1/14)	6.7% (1/15)	7.1% (1/14)	7.1% (1/14)	20.0% (3/15)
<b>UD–FP+</b>	0% (0/13)	7.7% (1/13)	0% (0/13)	0% (0/13)	7.7% (1/13)
<b>UD+FP–</b>	53.8% (7/13)	38.5% <sup>a</sup> (5/13)	46.2% (6/13)	16.7% (2/12)	15.4% (2/13)
<b>UD+FP+</b>	25.0% (3/12)	0% <sup>b</sup> (0/12)	25.0% (3/12)	8.3% (1/12)	0% (0/11)

544  
 545 Values with different letters (a, b) indicate differences between groups UD+FP– and  
 546 UD+FP+ ( $P < 0.05$ ).

547

548

## Figure legends

Figure 1: Changes of uterine size (score 1-6) in the first 65 days postpartum in dairy cows with and without uterine diseases (UD+/UD-) and with and without follicular puncture (FP+/FP-). Values are medians  $\pm$  median absolute deviation. Values with different letters (a, b) indicate differences between groups UD-FP- and UD-FP+ as well as between groups UD+FP- and UD+FP+ ( $P < 0.05$ ).

Figure 2: Changes of cervical diameter in the first 65 days postpartum in dairy cows with and without uterine diseases (UD+/UD-) and with and without follicular puncture (FP+/FP-). Values are medians  $\pm$  median absolute deviation. Values with different letters (a, b) indicate differences between groups UD-FP- and UD-FP+ ( $P < 0.05$ ).

Figure 3: Changes of diameter of the previously pregnant uterine horn in the first 65 days postpartum in dairy cows with and without uterine diseases (UD+/UD-) and with and without follicular puncture (FP+/FP-). Values are medians  $\pm$  median absolute deviation. Values with different letters (a, b) indicate differences between groups UD-FP- and UD-FP+ ( $P < 0.05$ ). At individual measuring times values with different letters are different ( $P < 0.05$ ).

Figure 4: Changes of plasma progesterone concentration in the first 56 days postpartum in dairy cows with and without uterine diseases (UD+/UD-) and with and without follicular puncture (FP+/FP-). Values are medians  $\pm$  median absolute deviation. Values with different letters (a, b) indicate differences between groups UD-FP- and UD-FP+ as well as between groups UD+FP- and UD+FP+ ( $P < 0.05$ ).

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575 Figure 5: Changes of total estrogen concentration in the first 56 days postpartum in  
576 dairy cows with and without uterine disease (UD+/UD-) and with and without  
577 follicular puncture (FP+/FP-). Values are medians  $\pm$  median absolute deviation.  
578 Values with different letters (a, b) indicate differences between groups UD-FP- and  
579 UD-FP+ ( $P < 0.05$ ).